OPTIMIZATION OF STATIN (SIMVASTATIN) BY *MONASCUS PURPUREUS* FTC 5356 IN SOLID-STATE FERMENTATION

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ABSTRACT

Monascus sp. is a non-pathogenic fungus that can produce statin called simvastatin that can lower blood cholesterol in human body. The objective of this research is to investigate the optimization condition of the simvastatin production in solid-state fermentation by *Monascus purpureus* FTC 5356. The local products that used as substrates were banana, guava, pumpkin, coconut meat, corn, papaya and white rice. The fermentation was conducted using the optimum condition of 50% initial moisture content, pH6 at 30°C for 12 days in order to obtain the best substrate. Among these local products, corn can produce the simvastatin while other five fruits do not produce simvastatin. Further experimental carried out using Central Composite Design (CCD) of Response Surface Methodology (RSM) by setting two parameters which are moisture content and nitrogen source by setting the lower and higher range for each of the parameters. From the analysis from RSM, there are 14 runs conducted to achieve the optimum condition to get the maximum production of simvasatin.

ABSTRAK

Monascus sp. ialah sejenis kulat bukan patogen yang boleh menghasilkan statin dikenali sebagai simvastatin yang boleh menurunkan kolesterol darah di dalam tubuh manusia. Objektif kajian ini adalah untuk mengkaji keadaan optimum penghasilan simvastatin dalam keadaan pepejal penapaian *Monascus purpureus* FTC 5356. Produk tempatan yang digunakan sebagai substrat ialah pisang, jambu, labu, kelapa , jagung, betik dan beras. Penapaian dijalankan menggunakan keadaan optimum yang awal ialah kandungan lembapan 50% l, pH6 pada suhu 30 ° C selama 11 hari untuk mendapatkan substrat yang terbaik. Antara produk tempatan, hasil menunjukkan jagung boleh menghasilkan simvastatin manakala lima jenis lagi buah-buahan tidak menghasilkan simvastatin. Kajian lanjut yang dijalankan menggunakan Design Pusat Komposit (CCD) Kaedah Tindakbalas Permukaan (RSM) dengan menetapkan dua parameter dengan kandungan kelembapan dan sumber nitrogen dengan menetapkan julat yang lebih rendah dan lebih tinggi bagi setiap parameter. Dari analisis dari RSM, terdapat 14 eksperimen dijalankan untuk mencapai keadaan yang optimum untuk mendapatkan pengeluaran maksimum simvasatin.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF RESEARCH

Statins is a group of drugs that used primarily in lowering blood cholesterol. Statin is generally capable in lowering cholesterol by 20 to 60 percent. The discovery of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A which is act as inhibitors called statin that was a breakthrough in the prevention of hypercholesterolemia and related diseases (Najma et al., 2010). As cardiovascular diseases related to high levels of cholesterol are among the main causes of death in our societies, there is a high incentive for developing processes for the production of statins, an FDA approved drug. All natural statins have a common molecular structure, a hexahydro-naphthalene system and a -hydroxy-lactone, but they differ from each other due to side chains and a methyl group around the ring (Gerardo et al., 2004). Statins also are fungal secondary metabolites and was the first enzyme in cholesterol biosynthesis (Manzoni et al., 2002).

Statins are available either in Tablet or capsule form, statin's are usually taken with dinner or bedtime. The results are typically evident after a period of four to six weeks of use. Medications in this group are usually easy to tolerate and cause few side effects (Najma et al., 2010). The mechanism that involved in controlling the production of plasma cholesterol

levels is the reversible inhibition of HMG-CoA reductase by the statins that is related to the structural similarity of the acid form of the statins to HMGCoA, the natural substrate of the enzymatic reaction (Manzoni et al., 2002).

The statins differ with respect to their ring structure and substituents. These differences in structure affect the pharmacological properties of the statins. Sometimes, statins have been grouped into two groups of statins according to their structure. Statins that belong to type 1 are pravastatin and simvastatin. Statins that are fully synthetic and have larger groups linked to the HMG-like moiety is often referred to as type 2 statins. Statins that belong to this group are atorvastatin and rosuvastatin (Najma et al., 2010). The biosynthetic pathway involved in statin production, starting from acetate units linked to each other in head to-tail fashion to form polyketide chains, has been elucidated by both early biogenetic investigations and recent advances in gene studies. Natural statins can be obtained from different general and species of filamentous fungi (Monzani et al., 2002).

There are five statins currently used as clinical use. Lovastatin and pravastatin (mevastatin derived) are naturally statins of fungal origin while simvastatin is semi-synthetic lovastatin derivative. Atorstatin and fluvastatin are synthetic statins, which derived from mevalonate and pyridine (Monzani et al., 2002).

Simvastatin and lovastatin are well-known hyperlipidemia and hypercholesterolemia drugs that act as cholesterol-lowering agents (Caron et al., 2007). Simvastatin (marketed under the trade names ZOCOR, SIMLUP, SIMCARD, and SIMVACOR) is metabolized to at least four primary metabolites, namely 6'β-OH simvastatin, 6'-exomethylene simvastatin, 6'β-hydroxymethyl metabolite, and 3"-OH simvastatin. After oral ingestion, simvastatin and lovastatin, which are inactive lactones, are hydrolyzed to the corresponding β-hydroxyacid form (Vickers et al., 1990a). This molecule is a principal metabolite and an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and ratelimiting step in the biosynthesis of cholesterol (Keon et al., 2010).

The metabolites resulting from microsomal oxidation of simvastatin and lovastatin by P450 enzymes are effective inhibitors of HMG-CoA reductase. Therefore, it has been suggested that the metabolites may contribute to the cholesterol-lowering effect of

simvastatin and lovastatin. However, systematic studies of the safety, efficacy, and toxicity of these metabolites have not been performed (Keon et al., 2010)

Lovastatin or also called Monacolin K is a potent drug for lowering blood cholesterol in human body. Lovastatin also a specific and a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), which is in the cholesterol biosynthesis, lovastatin act as a reductase that catalyzes the rate limiting step (Chang et al., 2002). Lovastatin also active to lower plasma cholesterol level in human and also animal, therefore it is the effective treatment for the patients that suffering hypercholesterolemia which is a primary risk for the artery disease (Frishman et al., 1989). Other research also indicated that lovastatin also indicated as a potential therapeutic agent for the various kind of tumors disease because lovastatin have ability to suppress the growth of the tumors (Chang et al., 2002).

Lovastatin can be extracted from the *Monascus sp*. especially *Monascus purpureus* by using several of substrates such as banana, papaya, guava, pumpkin, coconut meat, corn and also white rice. *Monascus sp*. is a non-pathogenic and widely used in Chinese foods and also as traditional Chinese medicine. *Monascus sp*. also extensively used in the food industry as a one of the colouring agent for the food such as red and also yellow pigment.

Lovastatin also have been investigated can therapeutically and can give an effective treatment also to prevent the diseases like atherosclerosis, sepsis, peripheral arterial disease, peripheral vascular disease, cerebro vascular disease, ischemic disease and bone fracture (Seraman et al., 2010). Lovastatin is extracted from the variety filamentous fungi for example *Monascus sp*. In particular monascus purpureus, monascus ruber and also monascus pilosus were found to be the most popular and also the most monascus used in production of lovastatin (Negishi et al., 1986).

Simvastatin is a compound derived from the natural lovastatin which is a secondary metabolites produced by filamentous fungus. The synthesis from lovastatin is a multistep process and has been intense interest because of its importance in the pharmaceutical industry.

Simvastatin a lactone analog of lovastatin which is used in the treatment of hypercholesterolemia. Simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, is administered in the form of lactone prodrug. Simvastatin lowers plasma cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase (Khaled, 2007)

Currently, two semisynthetic processes are widely used to synthesize simvastatin starting from lovastatin. One commonly adapted process starts with the hydrolysis of lovastatin to yield the key intermediate monacolin J, followed by the lactonization of the acid to protect the C11 hydroxyl group and trimethylsilylation protection of the C13 hydroxyl. The protected monacolin J is then subjected to acylation by dimethylbutyryl chloride to yield the protected form of simvastatin, which is subsequently deprotected to yield simvastatin. Both multistep processes are laborious, thus contributing to simvastatin being nearly five times more expensive than lovastatin. Therefore, a new semisynthetic scheme that can decrease the number of chemical transformations and increase the overall efficiency of the conversion can be of significant utility (Xinkai et al 2007).

For over thousands of years, the *Monascus sp.* was used on food which is called as Chinese traditional fermentation fungus. On the other hand, *Monascus sp.* also was very unique because either can extract to the lovastatin, monascus also can produces pigments like rubropunctatin (red colour), monascin (yellow colour), monascorubrin (red colour), anfaklavin(yellow colour), rubropunctamine (purple colour) and monascorubramine (purple colour) which now can be used to replace synthetic dyes by natural colourant and now already widely used (Manzoni et al.,1998; Chang et al.,2002). Statins currently available in different types and can classified also into natural statins which is can obtained directly by fermentation, semisynthetic and synthetic. Natural statins is like lovastatin and also pravastatin, while semisynthetic, atorvastatin and fluvastatin are synthetic statins (Manzoni and Rollini, 2002).

A variety of statins are available to lower plasma lipids to guideline levels, but all differ in their pharmacokinetic properties, drug interaction profiles, and risk of myotoxicity. This has been highlighted by the withdrawal of cerivastatin from the market as a result of serious safety concerns (Alberto, 2003).

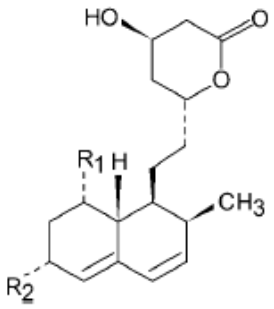

Figure 1.1: Base structure of statins - naphthalene ring and β-hydroxylactone (Manzoni et al., 2002)

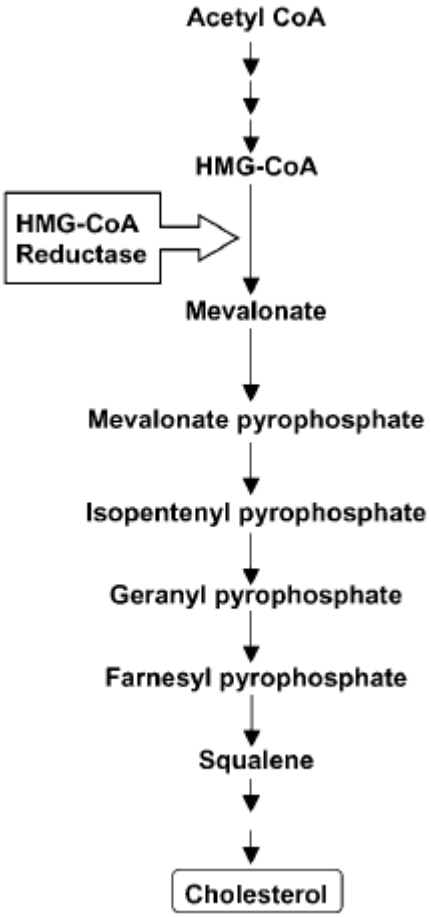

Figure 1.2 : Cholesterol biosynthetic pathway (Manzoni et al., 2002)

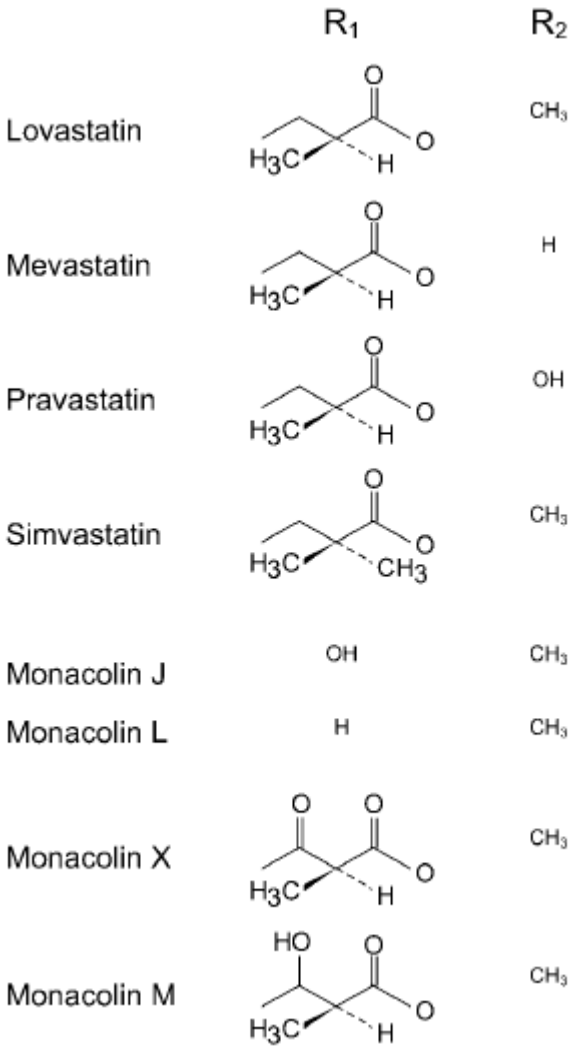

Figure 1.3 : Statin side chains linked at C8 (R1) and C6 (R2) of the base Structure (Manzoni et al., 2002)

1.2 PROBLEM STATEMENT

Nowadays, hypercholesterolemia is one of the world public health problem and also being the major cause of death in the western countries. Hypercholesterolemia is a primary risk factor for the country artery disease, heart attack and also stroke. Therefore, statins or also called blood-reducing cholesterol substance can inhibits the production of cholesterol by blocking of a key enzyme which is HMG-CoA reductase that activates cholesterol synthesis (Erdogrul and Azirak, 2004; Chen and Hu, 2005). In the industries, the production is the main focus, therefore industries need to produce high production but they also have to consume low. Therefore by using solid-state fermentation and also using local product as substrates which are banana, papaya, guava, pumpkin, coconut meat, corn and also white rice, therefore the production cost can be decreased. Statin such as lovastatin and simvastatin can produce by extraction of *Monascus purpureus* using solid-state fermentation. Solid-state fermentation is more advantages compared to submerged fermentation due to the substrate costs is little and also widely available. Solid-state fermentation also using less water and energy than submerged fermentation and the most important is in solid-state fermentation can yield higher production of lovastatin (Lian et al., 2006). Then, the ability of the *Monascus purpureus* to produce lovastatin in different solid substrates and also ability to produce high yield of lovastatin. The substrate chosen are banana, papaya, guava, pumpkin, coconut meat, corn and white rice.

1.3 RESEARCH OBJECTIVE

The purpose of doing this study is to achieve the objective which is to investigate the optimization condition of the statin production which is simvastatin in solid-state fermentation by *Monascus purpureus* by using local products which is banana, papaya, guava, pumpkin, coconut meat, corn and also white rice.

1.4 SCOPE OF RESEARCH

To achieve the objective for this experiment, there are few types of parameters has been identified which is first of all, the substrate selection. First of all, the first step is to obtain the best substrate from the seven local fruits. From the seven local products that been investigated, the best substrate chooses due to the two parameters that will be set which is percentage of nitrogen source added which is peptone and moisture content which being set under optimum moisture content which is 50%. Optimum amount of zinc sulphate also added which is 10%. All of the substrate was set under optimum condition which by using pH6 and setting under 30°C temperature inside incubator and left to undergo solid state fermentation for 11 days and the optimum days for the *Monascus purpureus* to obtain optimum yield is for 11 days. After get the best substrate, the investigated will be continued to study the effect of the moisture content and the concentration of the nutrient media added which is from nitrogen sources. Then, the experiment will continue to optimize the initial moisture of the substrate and the nutrient media which nitrogen sources is the best nutrient for simvastatin production. This study also using solid-state fermentation that is more advantages compared to the submerged fermentation.

1.4 RATIONALE AND SIGNIFICANCE OF RESEARCH

Statin is a very valuable product that can lowering the cholesterol in human body and already investigated and proven can be effective treatment of hypercholesterolemia and other major kind of diseases such as atherosclerosis, sepsis, peripheral arterial disease, cerebro vascular disease, ischemic disease and bone fracture (Seraman et al., 2010). In addition, statin now already been indicated for the therapeutic agent for the treatment of the variety kind of tumors because this statin have the ability to suppress tumors growth (Chang et al., 2002).

This research using local products which are banana, papaya, guava, pumpkin, coconut meat, corn and white rice as a substrate. Banana, guava, pumpkin and coconut meat never been use as a substrate from previous research. Therefore, this is an advantage if this research is success because it will become a new discovery in the science field.

From here we know that, simvastatin have a very valuable significant in the pharmaceutical industries. Therefore, hopefully this research can be commercialize and perhaps can increasing the pharmaceutical industries for our country.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

For over thousands of years, the *Monascus sp.* which is also called as Chinese traditional fermentation fungus was widely used on food and also been used as the essential part of wine production and for the other fermented foods (Seraman et al., 2010). From the cultivation of *Monascus sp.* on the rice grain, red mold rice which is also contains a large amount of γ-aminobutyric acid and also contain anti-hypertensive effects for human (S.Seraman et al., 2010). *Monascus sp.* also was very unique because either can extract to the lovastatin, *Monascus sp.* also can produces pigments like rubropunctatin (red colour), monascin (yellow colour), monascorubrin (red colour), anfaklavin(yellow colour), rubropunctamine (purple colour) and monascorubramine (purple colour) which now can be used to replace synthetic dyes by natural colourant and now already widely used (Manzoni et al.,1998; Chang et al.,2002).

The primary causes for the coronary artery disease is called hypercholesterolemia which also causes the major death in the western countries (Chang et al., 2002). Lovastatin is the best treatment for this disease. Statins currently available in different types and can classified also into natural statins which is can obtained directly by fermentation, semisynthetic and synthetic. Natural statins is like lovastatin and also pravastatin, while

semisynthetic, atorvastatin and fluvastatin are synthetic statins (Manzoni and Rollini, 2002). In the food processing, to add the aroma, nutrition and colour of the fermentation products, the *Monascus sp.* was commonly used because this species is such a non-pathogenic (Chang at el., 2002). Lovastatin also was first determined by Endo from the *Monascus ruber* and independently from *Aspergillus terreus* (Alberts et al.,1980). The first natural statin was from fungal secondary metabolite that being approved by the US Food and Drug Administration in August 1987 (Tobert 2003; Demain 1999; and Rollini 2002).

In the pharmaceutical study, after being experimented by three animal models, the study showed that this lovastatin can lower the blood cholesterol of hypercholesterolemia (Li et al., 1998). In addition, for the human clinical trial, the lovastatin showed a significant value in lowering cholesterol levels after tested in 83 tested individual (Heber et al., 1999).

2.2 STATIN

Statins are group of drugs that used primarily in lowering blood cholesterol. Statin are generally capable in lowering cholesterol by 20 to 60 percent. The discovery of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A which is act as inhibitors called statin that was a breakthrough in the prevention of hypercholesterolemia and related diseases (Najma et al., 2010). As cardiovascular diseases related to high levels of cholesterol are among the main causes of death in our societies, there is a high incentive for developing processes for the production of statins, an FDA approved drug. All natural statins have a common molecular structure, a hexahydro-naphthalene system and a -hydroxy-lactone, but they differ from each other due to side chains and a methyl group around the ring (Gerardo et al., 2004). Statins also are fungal secondary metabolites and was the first enzyme in cholesterol biosynthesis (Manzoni et al., 2002).

The most commonly prescribed drugs in medicine are statins. Statins significantly reduce the risk of heart attack and death in patient through clinical studies and proven that coronary artery disease (CAD) and also can reduce cardiac events in patients with high cholesterol levels who are at increased rosk for heart disease. Statin also was best known as drugs that can lower cholesterol also have several other beneficial effects that also may improve cardiac risk and that may turn out be even more important than their cholesterol reducing properties (Richard, 2011).

2.2.1 TYPE OF STATIN

Statins include well-known medications such as atorvastatin (Lipitor), simvastatin (Zocor), lovastatin (Mevacor), pravastatin (Pravachol), rosuvastatin (Crestor) and others. Lower cost generic versions of many statin medications are available (Mayo clinic, 2011).

The statins differ with respect to their ring structure and substituents. These differences in structure affect the pharmacological properties of the statins. Sometimes, statins have been grouped into two groups of statins according to their structure. Statins that belong to type 1 are pravastatin and simvastatin. Statins that are fully synthetic and have larger groups linked to the HMG-like moiety are often referred to as type 2 statins. Statins that belong to this group are atorvastatin and rosuvastatin (Najma et al., 2010).

There are five statins currently used as clinical use. Lovastatin and pravastatin (mevastatin derived) are naturally statins of fungal origin while simvastatin is semi-synthetic lovastatin derivative. Atorstatin and fluvastatin are synthetic statins, which derived from omevalonate and pyridine (Manzni et al., 2002). Lovastatin, simvastatin and pravastatin are derived from fungi. Simvastatin is chemically modified 2,2-dimethyll butyrate analogue of lovasatin. Provastatin then is a purified active metabolite of mevastatin with an open hydroxyl acid instead of lactone ring (Khaled, 2007).

The first representative of the new class of statin compounds was mevastatin which is derived from a strain of Penicilln citrinum. Lovastatin is a natural products while simvastatin is derived from the lovastatin. Pravastatin also derived from the natural products and fluvastatin is totally synthetic recemic mixture (Illingworth et al., 2001).

The fungal products lovastatin, pravastatin and simvastatin are structurally related since they have a hydronaphthalene in common and differ only at a few sites inhe molecule K shown in Figure 4 (Khaled, 2007).

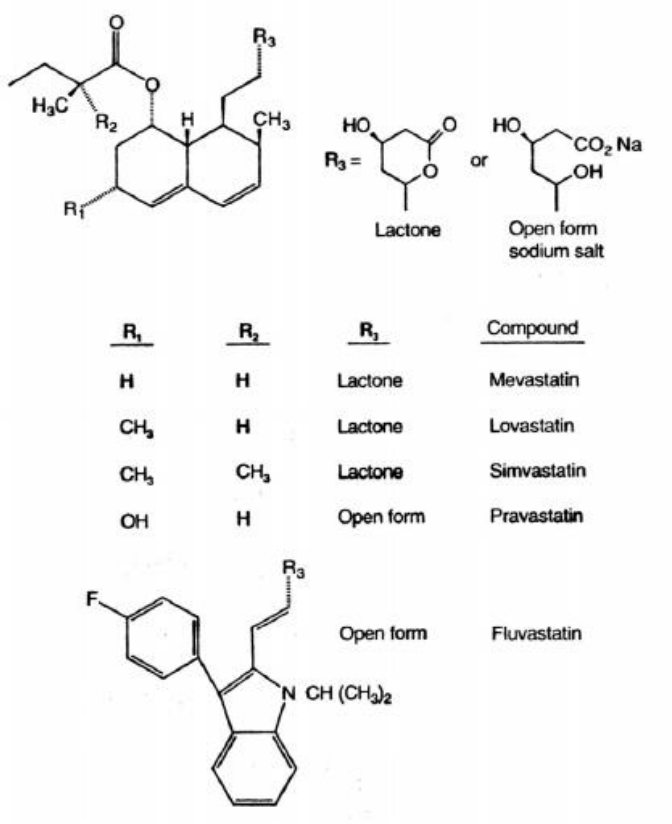

Figure 2.1 : Chemical structures of the main 3-hydroxy-3- methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (Khaled 2007; Desager and Horsmans, 1996)

Lovastatin, simvastatin, pravastatin and fluvastatin have similar pharmacodynamic properties. All can reduce LDL-cholesterol by 20 to 35%, a reduction which has been shown to achieve decreases of 30 to 35% in major cardiovascular outcomes. Simvastatin has this effect at doses of about half those of other 3 statins (Khaled, 2007).

2.2.2 BENEFIT OF STATIN

Statins is a group of drugs that used primarily in lowering blood cholesterol. Statin are generally capable in lowering cholesterol by 20 to 60 percent. The discovery of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A which is act as inhibitors called statin that was a breakthrough in the prevention of hypercholesterolemia and related diseases (Najma et al., 2010). As cardiovascular diseases related to high levels of cholesterol are among the main causes of death in our societies, there is a high incentive for developing processes for the production of statins, an FDA approved drug.

Lovastatin or also called Monacolin K is a potent drug for lowering blood cholesterol in human body. Lovastatin also a specific and a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), which is in the cholesterol biosynthesis, lovastatin act as a reductase that catalyzes the rate limiting step (Chang et al., 2002). Lovastatin also active to lower plasma cholesterol level in human and also animal, therefore it is the effective treatment for the patients that suffering hypercholesterolemia which is a primary risk for the artery disease (Frishman et al., 1989). Other research also indicated that lovastatin also indicated as a potential therapeutic agent for the various kind of tumors disease because lovastatin have ability to suppress the growth of the tumors (Chang et al., 2002).

Statin is a very valuable product that can lowering the cholesterol in human body and already investigated and proven can be effective treatment of hypercholesterolemia and other major kind of diseases such as atherosclerosis, sepsis, peripheral arterial disease, cerebro vascular disease, ischemic disease and bone fracture (Seraman et al., 2010). In addition, statin now already been indicated for the therapeutic agent for the treatment of the variety kind of tumors because this lovastatin have the ability to suppress tumors growth (Chang et al., 2002).

The four 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors used today are lovastatin, simvastatin, pravastatin and fluvastatin. The HMG-CoA reductase is the key enzyme of cholesterol synthesis. HMG-CoA reductase inhibitors are potent reversible inhibitors of this enzyme, which act by competing for the substrate HMG-CoA (Desager and Horsmans, 1996).

HMG-CoA reductase inhibitors are now widely used and account for the majority of prescriptions for lipid lowering drugs in many countries. HMG-CoA reductase inhibitors are the most effective agents developed to date for the treatment of patients with primary and secondary hypercholesterolaemia associated with increased levels of LDL cholesterol (Khaled, 2007).

The mechanism by which statins may influence prostate cancer is unknown but may involve their cholesterol-lowering properties or their influence on other pathways (Allison et al., 2010). Nowadays, recent evidence suggests that statins may also act as chemoprotective agents against various types of cancers (Vikram et al., 2007). Then, the statins have been reported to protect against stroke events in stroke-prone spontaneously-hypertensive rats and

ameliorate stroke severity by inhibition of superoxide production and modulation of inflammation in the brain (Sung et al., 2004).

2.2.3 EFFECT OF STATIN

The hypocholesterolemic effects of statins are evident after only a few days of therapy. Lovastatin, simvastatin, and pravastatin are well tolerated drugs; at 40 mg lovastatin a mean reduction of 30% in total plasma cholesterol, 40% in LDL (low-density lipoprotein), 35% in VLDL (very low-density lipoprotein) cholesterol, and 25% in triglycerides, and an increase of 10% high density lipoprotein (HDL)-cholesterol was observed (Monzani et al., 2002; Tobert 1987).

The results reported since 1987, the year of approval of lovastatin as a therapeutic drug by the FDA, indicate that statins can be employed successfully in the treatment of hypercholesterolemia. However, the benefit-risk relationship must always be taken into account. The marked lipid-lowering effects of statins have led to a substantial reduction in coronary events, as revealed by clinical, epidemiological, and pathological studies (Monzani et al.,2002; Chong et al. 2001; Farnier and Davignon 1998; Furberg 1999; Maron et al. 2000).

In addition to reducing the risk of cardiovascular morbidity and mortality, statins can prevent stroke and reduce the development of peripheral vascular disease (Monzani et al., 20020; Maron et al. 2000).

Many years, have proven beyond reasonable doubt that virtually all patients at cardiovascular risk benefit from effective lipid-lowering therapy with statins, even those with normal LDL cholesterol levels (Alberto, 2003). Based on these recent trials, patients with coronary heart disease (CHD), diabetes, the elderly, menopausal women, recipients of donor organs or those with HIV are at highest absolute cardiovascular risk, even though LDL cholesterol levels are not always elevated, and have the most to gain from statin therapy. In addition, many patients with mixed dyslipidaemia are at increased cardiovascular risk as a result of low High Density Lipoprotein (HDL) cholesterol and high triglycerides, and/or abnormal small, dense atherogenic Low Density Lipoprotein (LDL) cholesterol levels (Alberto, 2003).

Statin therapy reduces the incidence of coronary events in part by slowing the progression of atherosclerosis, with coronary angiography studies consistently demonstrating the ability of statin treatment to slow the CHD progression (Alberto, 2003).

Therefore statin treatment suggested to provide clear outcome benefits in patients with average cholesterol levels. These findings suggest that the reduction in coronary risk caused by statin therapy may reflect actions of this drug class independent of their lipid lowering effects (Alberto, 2003).

2.3 APPLICATION OF STATIN

2.3.1 LOVASTATIN

Potent competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) is called as lovastatin which is also known as mevinolin, monacolin K and also mevacor. HMG-CoA is reductase which is rate-limiting enzyme in cholesterol biosynthesis (Chang et al., 2002). Other than that, lovastatin now already been indicated for the therapeutic agent for the treatment of the variety kind of tumors because this lovastatin have the ability to suppress tumors growth (Chang et al., 2002). Lovastatin also have been investigated can therapeutically and can give an effective treatment also to prevent the diseases like atherosclerosis, sepsis, peripheral arterial disease, peripheral vascular disease, cerebro vascular disease, ischemic disease and bone fracture (Seraman et al., 2010).

Lovastatin is extracted from the variety filamentous fungi for example *Monascus sp.* In particular *Monascus purpureus, Monascus ruber* and also *Monascus pilosus* were found to be the most popular and also the most *Monascus sp.* used in production of lovastatin (Negishi et al., 1986). Other than that, also indicated that lovastatin also indicated as a potential therapeutic agent for the various kind of tumors disease because lovastatin have ability to suppress the growth of the tumors (Chang et al., 2002). Lovastatin also active to lower plasma cholesterol level in human and also animal, therefore it is the effective treatment for the patients that suffering hypercholesterolemia which is a primary risk for the arthery disease (Frishman et al., 1989).

Recently, since the death because of the heart disease increasing due to the one of the famous factor which is hypercholesterolemia and because of that lovastatin and it

semisynthetic derivatives became the important natural drugs which is from the natural sources that is *Monascus sp*. (Wei et al., 2007). According to the history, lovastatin was firstly being isolated by Endo from *Monascus ruber* and then independently by the Alberts et al., 1980 from *Aspergillus terreus* (Chang et al., 2002).

2.3.2 SIMVASTATIN

Simvastatin is a water insoluble drug used as a hypocholestrolemic agent (Jaleh et al., 2011). Simvastatin is a compound derived from the natural lovastatin which is a secondary metabolites produced by filamentous fungus. The synthesis from lovastatin is a multistep process and has been intense interest because of its importance in the pharmaceutical industry.

Simvastatin a lactone analog of lovastatin which is used in the treatment of hypercholesterolemia. Simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, is administered in the form of lactone prodrug. Simvastatin lowers plasma cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase (Khaled, 2007).

Currently, two semisynthetic processes are widely used to synthesize simvastatin starting from lovastatin. One commonly adapted process starts with the hydrolysis of lovastatin to yield the key intermediate monacolin J, followed by the lactonization of the acid to protect the C11 hydroxyl group and trimethylsilylation protection of the C13 hydroxyl. The protected monacolin J is then subjected to acylation by dimethylbutyryl chloride to yield the protected form of simvastatin, which is subsequently deprotected to yield simvastatin. Both multistep processes are laborious, thus contributing to simvastatin being nearly five times more expensive than lovastatin. Therefore, a new semisynthetic scheme that can decrease the number of chemical transformations and increase the overall efficiency of the conversion can be of significant utility (Xinkai et al 2007).

Simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, is administered in the form of lactone prodrug. The lactone ring is hydrolyzed *in vivo* to produce the hydroxyl acid derivatives which are the pharmacologically active forms of this drug, and this is believed to take place predominantly in the liver (Khaled, 2007).

2.4 PRODUCTION OF STATIN BY *MONASCUS PURPUREUS*

2.4.1 PRODUCTION OF SIMVASTATIN AND LOVASTATIN

For centuries, *Monascus sp.* has been used widely in Asia as a coloring of fish, Chinese cheese, red wine and sausages (Pattanagul et al., 2008). *Monascus sp.* widely used long time ago as a folk medicine for the food digestion and also blood circulation and also as a treatment of other sickness (Panda at el., 2010). *Monascus sp*. belong to the *Ascomycetes* group and family of *Monascaceae. Monascus purpureus* easily can be distinguished by it ascospores which is in spherical shape (Pattanagul et al., 2007). *Monascus sp*. are non-pathogenic and use in the food processing to obtain the aroma, nutrition and also colour of the fermentation products (Chang et al., 2002). Using *Monascus sp.* for the production of the lovastatin indicate that give advantageous with an increased saving in cost and if using directly as a functional food as long it is proves to be nontoxic (Xu et al., 2005). Various of active ingredients including lovastatin owned by the *Monascus purpureus* and several trials been done for its ability toward lowering the lipid have been conducted (Liu et al., 2006).

Simvastatin is a methyl analogue of lovastatin and is synthesized from a fermentation product of *Aspergillus terreus* (Khaled 2007; Hoffman *et al*., 1986). Simvastatin is a nonhygroscopic white crystalline powder, insoluble in water but quite soluble in chloroform, methanol and alcohol (Mauro, 1993) with pKa of 4.68 (Corsini *et al.,* 1999). The molecular weight of this compound C25H38O5 is 418.57. Simvastatin is the pharmacologically inactive lactone form of simvastatin acid, butanoic acid, 2,2-dimethyl-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl) ethyl]-1-naphthalenyl ester. Simvastatin is a lactone which needs the opening of the ring for it to become active.

Simvastatin is a crystalline powder, that practically water insoluble that is obtained as a fermentation product of *Aspergills terreus* and is poorly absorbed from the gastro-intestinal (GI) . Therefore, it is very important to enhance its dissolution rate substantially leading to its improved bioavailability (Jaleh et al., 2011).

2.4.2 CONNECTION BETWEEN LOVASTATIN AND SIMVASTATIN

Simvastatin is a semisynthetic derivative of the fungal polyketide lovastatin and is an important drug for lowering cholesterol levels in adults (Xinkai et al., 2007). The synthesis of simvastatin from lovastatin is a multistep process and has been of intense interest because of its importance in the pharmaceutical industry (Xinkai et al., 2007).

Currently, two semisynthetic processes are widely used to synthesize simvastatin starting from lovastatin. One commonly adapted process starts with the hydrolysis of lovastatin to yield the key intermediate monacolin J, followed by the lactonization of the acid to protect the C11 hydroxyl group and trimethylsilylation protection of the C13 hydroxyl. The protected monacolin J is then subjected to acylation by dimethylbutyryl chloride to yield the protected form of simvastatin, which is subsequently deprotected to yield simvastatin. Both multistep processes are laborious, thus contributing to simvastatin being nearly five times more expensive than lovastatin. Therefore, a new semisynthetic scheme that can decrease the number of chemical transformations and increase the overall efficiency of the conversion can be of significant utility (Xinkai et al 2007).

Parameter	Lovastatin	Simvastatin	Pravastatin	Fluvastatin	Atorvastatin	Cerivastatin
Prodrug	Yes	Yes	No	No	No	No
Crosses blood brain barrier	Lactone	Lactone	No	No	N.A	N.A.
Lipophilicity	Lipophilic	Lipophilic	Hydrophilic	Hydrophilic	Lipophilic	N.A.
Oral pharmacokinetics						
• Dose (mg/day)	20-8	10-40	20-4	20-80	2.5-80	0.1-0.3
• Absorption (%)	30	60-85	35	98	N.A.	N.A.
• Bioavailability (%)	< 5	< 5	10	10-35	12	60
• Effect of food	↑ 50%	No	↓ 30%	↓ 15-25%	↓ 13%	↓23%
T_{max}	2-6	1.3-2.4	0.9-1.6	0.5-1.5	2-4	0.5-4
Terminal half-life (hr)	2.5-15	1.9-15.6	1.3-2.6	0.5-3.1	14	1.7-2.7
Hepatic extraction (%)	62-69	>78	46	68	N.A.	N.A.
Renal elimination (%)	30	13	20-60	6	< 2	30
Protein binding (%)	> 90	> 90	43-48	95-98	> 95	N.A.
p-Glycoprotein substrate	Yes	N.A.	Yes	Yes	N.A.	N.A.
CYP substrate	CYP3A	CYP3A	No	CYP2C9	CYP3A	CYP3A
Metabolites effect	Yes	Yes	No	No	Yes	Yes
Mostly eliminated as	Metabolites	Metabolites	Unchanged	Metabolites	N.A.	Metabolites

N.A., not available

Table 2.1: Comparison of HMG-CoA Reductase inhibitors (Khaled, 2007;Christians *et al.*, 1998). The Table show only lovastatin and simvastatin are prodrug and crosses blood drain brain barrier.

2.5 SOLID STATE FERMENTATION

Solid substrate fermentation was done by mixed cultures of different fungal. For better biomass and also secondary metabolite productions, the co culture of fungi during the fermentation process. To enhance enzyme, organic acid production and also microbial bioconversion reaction, there were several reports showed (Banerjee et al., 2005; Pandey et at., 1999; Temudo et al. 2007).

By using different process parameter that can contributing the lovastatin production, the optimum levels was identified, which is carried out the solid state fermentation in conical flasks that contain optimized nutrients (Panda et al., 2008). There are four process parameters been used which is temperature, fermentation time, inoculums volume and pH of the solid medium were chosen for investigating and also the procedure of this process parameters was mostly contribute to the growth of the different fungal strain during solid state fermentation (Panda et al., 2008).

In solid-state fermentation (SSF), the cultivation of *Monascus sp*. in steamed rice is very exuberant. Carbon and nitrogen give an effect to the production of lovastatin and for the fungus growth because there are many natural substrates that showed similar or even higher quantities of carbohydrate and protein because these two nutrients contribute to the production of lovastatin (Soccol et al., 2003).

Nowadays, the solid-state fermentation become the most effective ways to ferment *Monascus sp*. to gain the lovastatin because of its advantages compared to the submerged fermentation. This advantages are widely available, water and also energy is less used and the most important fact is it is can produce high yield of the lovastatin (Wei et al., 2007). For addition, to minimize the production cost, a few efforts have been done using solid-state fermentation for the production of the lovastatin (Szakacs et al., 1998). The capability of fungus like *Monascus sp*. to produce lovastatin in the variety of solid substrates is investigated (Jaivel and Marimuthu, 2010).

2.5.1 EFFECTS OF SUBSTRATES

In the solid state fermentation focused on *Monascus sp*. fermentation, such substrate might showed a potential substrate and gives the best result in the production of other metabolites in solid-state fermentation (Soccol et al., 2003).

There also a report that describe some other raw materials used as a substrate for the *Monascus sp*. growth which is cassava starch, pear juice and also dairy milk. There are also supplement this substrate with the others nutrients such as vitamins and also organic nitrogen supplements (Carvalho et al., 2006). Substrates such as wheat bran, rice bran, maize flour and sorghum grain being used in the solid-state fermentation process to find the suiTable substrate for maximum lovastatin production then incubated to get the yield by the HPLC analysis (Morovjan et al., 1997).

Important factor that affect fungi growth and productivity is the composition of a solid substrate. Rice is a common substrate and soy-bean flour is a common additive substrate for the SSF of M.ruber (Xu et al., 2005). The culture medium has significant influence on the production of a metabolite as with any solid state fermentation product. The important in the industrial scale fermentation in about the screening and also optimization of the substrate constituents. The important thing for the lovastatin production is the selection

and also the composition of the nutrients of a suiTable substrates from the SSF (Xu et al., 2005).

2.5.2 EFFECT OF CARBON AND NITROGEN ADDITIVES

Carbon and nitrogen sources is very important in the fermentation activity because this nutrients is contribute and gives major effect to the formation of biomass and the metabolite (Xu et al., 2005). Peptone is one of the organic nitrogen source for the lovastatin production, the lower concentration of the peptone will be increase the production of lovastatin but the higher the concentration of the peptone will decreasing the production of the lovastatin. The effect of the carbon source such as glucose, maltose and also glycerol also the combination either both of the carbon source will required for the higher of the production of the lovastatin (Miyake et al., 2006).

2.6 ANALYSIS USING HIGH PERFORMANCE LIQUID CHROMATOGRAOHY (HPLC)

Analysis is carried out using high performance liquid chromatographic (HPLC) in an reverse phased C_{18} column (Morovjov et al.,1997). Using HPLC, lovastatin was quantified as β-hydroxy acid form which is unstabble and freshly prepared from lactone form (Friedrich et al., 1995). Using Rheoyne injector of 20µl manually, the binary gradient system was used and the samples injected. The mobile used were acetonitrile and 0.1% orthophosphoric acid in water in the ratio of 60:40 by the flow rate of 1.5 ml min^{-1} (Seraman et al., 2010). Nowadays, for HPLC analysis already processed in the same manner done in the previous study for the preparation of the sample and the standard solution of the HPLC (Xu et al., 2005).

2.7 EXPERMENTAL DESIGN AND OPTIMIZATION BY RESPONSE SURFACE METHODOLOGY (RSM)

Optimization using response surface methodology (RSM) has been widely used for various phases in the fermentation process using various parameters (Panda et al., 2009). Using RSM the researcher can reduce the experimental because RSM is a very systematic technique for testing multiple process variables compared to the study of a variable in one time and such technique also can be determined and quantified for the interaction between variables (Chang et al., 2002). Researcher also can get their optimum result because RSM